STUDIES ON THE UTILIZATION OF CARBON SOURCES BY THERMOPHILIC ACTINOMYCETES

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The utilization of different carbon sources has been the object of several studies (Salzman, 1901; Münter, 1913; Lieske, 1921; Cochrane and Hawley, 1956).

In the present paper the utilization of carbon sources by strains of thermophilic actinomycetes belonging to different species is reviewed.

Materials and methods

From the carbohydrates serving as carbon sources some representatives of the mono-, oligo-, and polysaccharides (such as D-glucose, D-arabinose, D-xylose, maltose, and starch) were considered. The decomposition of cellulose has been studied in a previous work (Balla, 1968). For experimental purposes a synthetic culture medium of standard composition was used as this medium contained no other organic substance than the carbohydrate under investigation. The basic culture was prepared according to Waksman (1961), but it was supplemented with 1% of the carbohydrate to be investigated. The cultures were kept in 100 ml Erlenmeyer flasks. In view of its carbohydrate content the culture medium was sterilized by the fractionation process. The sterile culture media inocculated with monospore cultures of the strains investigated were then incubated at 37° and 50°C, respectively. The incubation time varied according to both the physiological properties of the strains and the type of the carbohydrate serving as substrate. A stop in the growth of the colony diameter indicated the end of incubation.

For measuring the growth indicating the extent of carbon utilization two methods were used:

(1) measuring growth of the colony diameter, and
(2) dry weight measurement.

Both methods have been described in detail by Horváth (1966).

Starch decomposition was investigated by both qualitative and quantitative methods. Qualitative estimation of amylase was carried out on starch-

agar plates at two different temperatures (37° and 50°C). At the end of incubation (72 hrs) the presence of extracellular enzyme was demonstrated by means of an iodine-starch colour reaction, and the dimension of the decomposition zone was measured at the two different incubation temperatures. With strains with an intensive activity as judged by the qualitative method starch decomposition was tested quantitatively as well. For these tests a liquid culture medium containing 5 g starch/liter was used. For five days the extent of starch decomposition was checked by daily sampling. The total amount of sugar obtained from starch by this method was then estimated spectrophotometrically.

Composition of the reagent:

6% orcin solution
60% H_2SO_4
96% ethanol

96% ethanol served to precipitate non-decomposed starch in the sample. 60% H_2SO_4 complete by hidrolized the carbohydrates formed during starch decomposition. In acidic medium, the mono-saccharides gave a colour reaction with orcin, the colour was measured in an UVIFOT spectrophotometer at 540 mμ. Non-inocculated media served as a control. The extinction values obtained were then converted – by means of standard curves obtained with samples containing a known amount of starch – into total of sugar values (mg/ml).

Results and conclusions

For strains belonging to different species the utilization of various carbon sources was investigated at two incubation temperatures (37° and 50°C). The following strains of the corresponding species were used:

TA_1	=	*Streptomyces thermoviolaceus* ssp. *apingens*
$TA_{4, 6, 60, 81}$	=	*Thermoactinomyces thermophilus*
$TA_{11, 29}$	=	*Streptomyces thermodiastaticus*
$TA_{5, 77}$	=	*Streptomyces thermofuscus*
$TA_{16, 36}$	=	*Thermopolyspora polyspora*
TA_{42}	=	*Streptomyces thermovulgaris*
$TA_{44, 74}$	=	*Thermomonospora lineata*
TA_{49}	=	*Thermoactinomyces vulgaris*

The increase in colony diameter was measured; at the end of the incubation period the dry weight was determined. These data as well as those indicating utilization rate of the carbon source – these latter being characterized by the daily increase in dry weight – are listed in Table I. The results obtained stand for the average values belonging to four parallel cultures.

Table I.

Utilization of carbon sources by actinomycetes strains

Strain No.	Carbohydrates	Colony diam. (mm)		Dry weight (mg)		Incubation (day)		Utilization rate	
								dry wt	mg/day
		37 °C	50 °C	37 °C	50 °C	37 °C	50 °C	37 °C	50 °C
TA_1	starch	12.0	15.0	5.8	7.5	10	12	0.58	0.7
	glycose	8.5	12.5	2.0	6.0	14	9	0.1	0.6
	maltose	22.1	16.1	8.2	1.0	19	13	0.4	0.08
	xylose	16.0	3.2	1.0	1.0	16	8	0.06	0.1
	arabinose	17.0	2.8	6.3	1.0	12	10	0.5	0.1
TA_4	starch	9.0	17.0	5.94	11.2	12	10	0.94	1.12
	glycose	13.5	18.0	9.0	6.6	16	11	0.5	0.6
	maltose	18.8	16.6	9.2	5.5	17	13	0.5	0.4
	xylose	14.2	2.4	11.6	1.0	15	7	0.7	0.1
	arabinose	20.2	1.7	1.0	—	15	7	0.06	—
TA_5	starch	12.0	17.0	8.4	11.9	11	12	0.76	0.99
	glycose	18.9	19.3	13.0	12.2	16	13	0.8	0.9
	maltose	19.5	18.3	12.0	8.0	19	13	0.6	0.6
	xylose	18.9	1.4	6.0	1.0	15	10	0.4	0.1
	arabinose	20.1	2.1	4.0	1.0	27	6	0.1	0.1
TA_{11}	starch	11.0	18.0	8.25	10.5	12	11	0.68	0.95
	glycose	20.1	12.7	3.7	3.0	12	9	0.3	0.3
	maltose	20.4	18.7	8.5	1.0	19	13	0.4	0.08
	xylose	12.2	3.1	9.3	1.0	17	8	0.5	0.1
	arabinose	16.5	1.7	3.6	—	17	6	0.2	—
TA_{16}	starch	13.0	11.0	20.8	17.6	10	10	2.08	1.76
	glycose	18.0	15.4	29.0	4.5	12	10	2.4	0.45
	maltose	19.0	7.4	11.0	2.0	11	14	1.0	0.1
	xylose	7.0	—	—	—	16	7	—	—
	arabinose	21.4	15.9	—	—	16	11	—	—
TA_{42}	starch	10.0	26.0	3.3	8.58	10	10	0.33	0.86
	glycose	2.3	6.0	—	2.0	14	7	—	0.2
	maltose	3.0	7.3	5.0	1.0	17	14	0.3	0.07
	xylose	3.0	—	—	—	8	7	—	—
	arabinose	2.4	1.3	—	—	6	6	—	—
TA_{44}	starch	12.0	25.0	2.4	5.0	12	10	0.2	0.5
	glycose	5.1	5.6	1.0	1.0	10	6	0.01	0.1
	maltose	—	—	—	—	14	14	—	—
	xylose	—	—	—	—	7	7	—	—
	arabinose	—	—	—	—	6	6	—	—
TA_{49}	starch	10.0	16.0	5.4	8.64	10	8	0.54	1.08
	glycose	15.2	21.1	15.0	11.0	11	6	1.3	1.8
	maltose	8.2	7.4	1.0	1.0	13	13	0.07	0.07
	xylose	—	—	—	—	8	8	—	—
	arabinose	24.1	21.3	1.0	3.0	16	11	0.09	0.2

It is obvious that the growth in colony diameter does not reflect unambiguously the development of actinomycetes and utilization of the nutrient, Therefore, the extent of utilization of the individual carbon sources has been established as a function of the increase in dry weight (cf. Table II).

Table II.

Order of utilization of the different carbon sources

Strain No.	I. Starch		II. Glucose		III. Maltose		IV. Xylose		V. Arabinose	
	37 °C	50 °C	37 °C	50 °C	37 °C	50 °C	37 °C	50 °C	37 °C	50 °C
TA_1	0.58	0.7	0.1	0.6	0.4	0.8	0.06	0.1	0.5	0.1
TA_4	0.49	1.12	0.5	0.6	0.5	0.4	0.7	0.1	0.06	–
TA_5	0.76	0.99	0.8	0.9	0.6	0.6	0.4	0.1	0.1	0.1
TA_{11}	0.68	0.95	0.3	0.3	0.4	0.08	0.5	0.1	0.2	–
TA_{16}	2.08	1.76	2.4	0.45	1.0	0.1	–	–	–	–
TA_{42}	0.33	0.86	–	0.2	0.3	0.07	–	–	–	–
TA_{44}	0.2	0.5	0.01	0.1	–	–	–	–	–	–
TA_{49}	0.54	1.08	1.3	1.8	0.07	0.07	–	–	0.09	0.2

Table 2 demonstrates that four strains belonging to different species the order of utilization of carbon sources is as follows:

starch $\approx$ glucose $>$ maltose $>$ xylose $>$ arabinose.

Strains marked $TA_{6, 29, 36, 42, 60, 74, 77, 81}$ were used for a more detailed study of starch decomposition.

For starch decomposition the results obtained by qualitative tests (on the basis of the decomposition circle diameter) are listed in Table III. Amylase activity of the strains investigated was tested at three different temperatures (20°, 37°, and 50°C) for five days. Table 4 demonstrates the average values (mg/ml) of the total amounts of sugar obtained with parallel cultures as calculated from the extinction-, and standard curves, respectively.

Table III.

Results of qualitative test on starch decomposition

Strain No.	Colony diameter (cm)		Decomposition circle diameter (cm)	
	37 °C	50 °C	37 °C	50 °C
TA_6	1.0	1.4	1.5	1.0
TA_{29}	1.0	1.3	1.2	1.7
TA_{36}	1.1	1.4	0.9	1.1
TA_{42}	1.0	2.6	1.0	1.6
TA_{60}	0.8	1.2	0.5	0.9
TA_{74}	0.7	1.1	0.5	0.9
TA_{77}	1.0	1.2	0.8	1.4
TA_{81}	1.2	1.6	0.3	1.1

Plots of amylase activity of the strains investigated as a function of both the total amount of sugar (mg/ml) and the incubation time, yielded the following results:

(1) With 3 strains ($TA_{6, 74, 81}$) amylase activity was considerably higher at 50° than at 37°C (cf. Fig. 1).

Table IV.

Results of qualitative tests on starch decomposition

Strain No.	Temperature C°	Average extinction values					Average values of total amount of sugar mg/ml				
		24h	48h	72h	96h	120h	24h	48h	72h	96h	120h
TA_6	20	–	–	–	–	–	–	–	–	–	–
	37	0.04	0.13	0.56	0.57	0.49	0.100	0.325	1.40	1.425	1.225
	50	0.04	0.63	0.72	0.50	0.26	0.100	1.575	2.050	1.250	0.650
TA_{29}	20	0.07	0.11	0.31	0.29	0.43	0.175	0.250	0.775	0.725	1.075
	37	0.13	0.43	0.65	0.77	0.95	0.325	1.075	1.625	1.925	2.375
	50	0.06	0.45	0.66	0.67	1.02	0.150	1.125	1.650	1.675	2.550
TA_{36}	20	–	–	–	–	–	–	–	–	–	–
	37	0.13	0.62	0.65	0.65	0.67	0.375	1.575	1.625	1.625	1.675
	50	0.16	0.53	0.57	0.56	0.55	0.400	1.325	1.425	1.400	1.375
TA_{42}	20	–	–	–	–	–	–	–	–	–	–
	37	0.02	0.57	0.79	0.84	0.93	0.050	1.425	1.975	2.100	2.325
	50	0.67	0.79	0.76	0.83	0.92	1.675	1.975	1.900	2.075	2.300
TA_{60}	20	–	–	–	–	–	–	–	–	–	–
	37	0.08	0.17	0.96	1.14	0.92	0.200	0.425	2.400	2.850	2.300
	50	0.37	0.57	0.92	0.61	0.70	0.925	1.425	2.300	1.525	1.750
TA_{74}	20	–	–	–	–	–	–	–	–	–	–
	37	0.21	0.38	0.96	0.58	0.76	0.525	0.950	2.400	1.450	1.900
	50	0.31	0.88	1.12	0.78	0.41	0.775	2.200	2.800	1.950	1.025
TA_{77}	20	–	0.37	0.06	0.01	0.01	–	0.925	0.150	0.025	0.025
	37	0.21	1.1	0.85	0.46	0.69	0.525	2.775	2.125	1.150	1.725
	50	0.33	0.88	0.94	1.13	0.61	0.825	2.200	2.350	2.825	1.525
TA_{81}	20	–	–	–	–	–	–	–	–	–	–
	37	–	0.26	0.56	0.94	0.79	–	0.650	1.400	2.350	1.975
	50	0.17	1.19	0.92	0.73	0.61	0.425	2.975	2.300	1.825	1.525

(2) With 3 strains ($TA_{29, 42, 77}$) amylase activity was the same at both incubation temperatures (Fig. 2).

(3) With 2 strains ($TA_{36, 60}$) amylase activity proved to be lower at 50° than at 37°C (cf. Fig. 3).

A comparison of the results obtained for amylase activity by the two different methods (decomposition circle diameter-, and spectrophotometric measurements, respectively) indicates the following:

(1) With two strains ($TA_{74, 81}$) according to both methods both amylase activity and the extent of starch decomposition were higher at 50° than 37°C (cf. Tables 3 and 4).

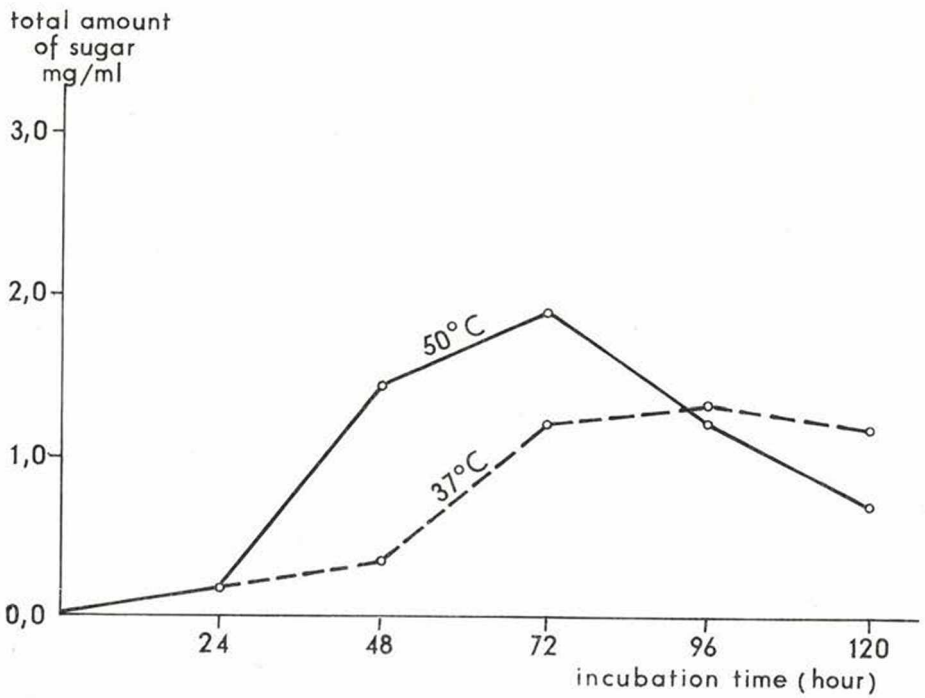

Fig. 1. Amylase activity of strain TA_6

(2) With three strains ($TA_{29, 42, 77}$), according to the decomposition circle diameter the extent of starch decomposition varied at the two different temperatures, whereas according to quantitative measurements amylase activity proved to be approximately the same (Figs. 2, 4 and 5).

(3) With two strains ($TA_{36, 60}$) the decomposition circle diameter indicated a greater extent of starch decomposition at 50°C; according to quantitative measurements, however, amylase activity was higher at 37°C (Figs. 3, 6 and 7).

(4) With one strain (TA_6) according to qualitative tests the extent of starch decomposition was higher at 37°C, whereas quantitative measurements revealed a higher amylase activity at 50°C (Figs. 1, 8 and 9).

From the experimental results it can be concluded that:

(1) Most of the strains investigated utilized D-glucose, maltose and starch, respectively, to practically the same extent. This is in good agreement with the data reported by G o t t l i e b and P r i d h a m (1948) for common actinomycetes.

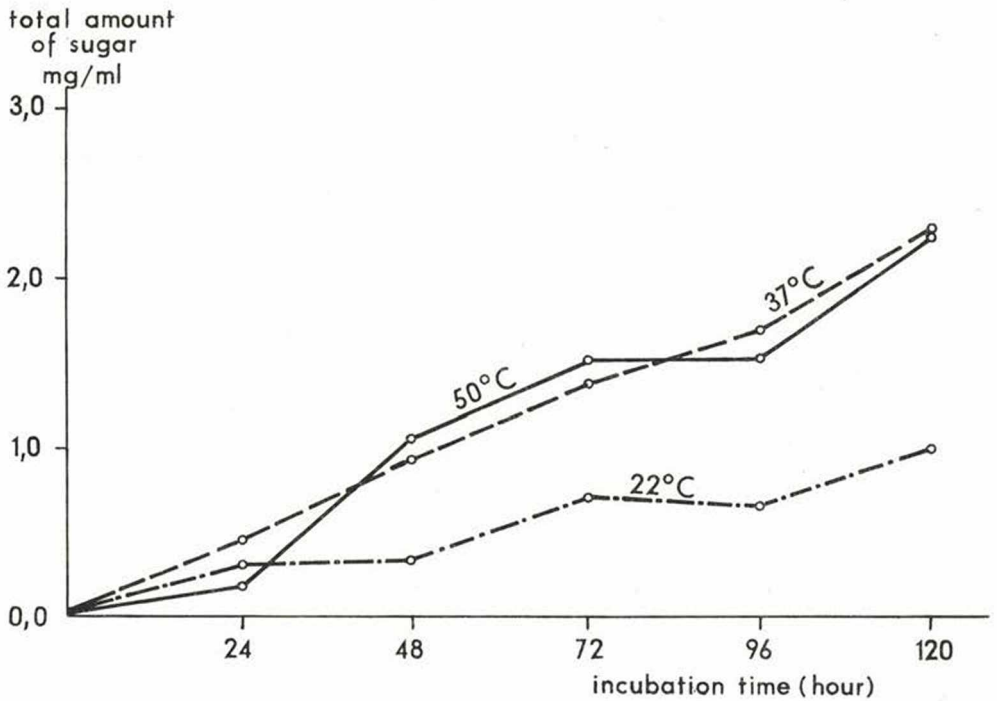

Fig. 2. Amylase activity of strain TA_{29}

(2) On the other hand, in the case of cellulose, xylose, arabinose (rhamnose, raffinose, lactose and mannose) the utilization selective. Consequently, from the point of view of systematization these carbohydrates can be considered as significant.

(3) For common actinomycetes, K r a i n s k y (1914) and W a k s m a n (1920) have investigated the utilization of carbohydrates from the point of view of systematization. It is of interest to mention that most of the actinomycetes investigated by the above authors can not utilize arabinose as a carbon source.

(4) The experimental results on starch decomposition clearly show that the enzymatic hydrolysis of starch is greatly dependent on the incubation temperature.

(5) A comparison of the results obtained on starch decomposition by qualitative and quantitative methods, respectively, decisively point to the importance of accurate measurements.

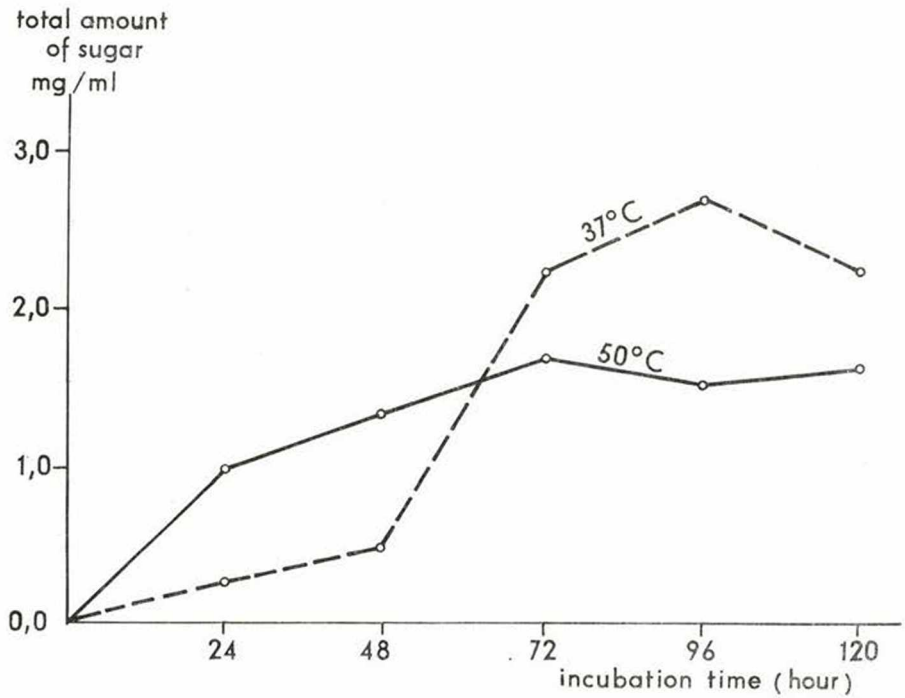

Fig. 3. Amylase activity of strain TA_{60}

Summary

The utilization of carbon sources by thermophilic actinomycetes strains belonging to different species was investigated. Dry weight measurement offers an unambiguous and reliable method for determining the extent of carbon utilization. Among the carbohydrates investigated from the point of view of systematizing, cellulose, arabinose, and xylose proved to be significant. The results obtained by more thorough examination of starch decomposition decisively point to the importance of accurate qualitative and quantitative measurements and verify the incubation temperature dependence of enzymatic hydrolysis.

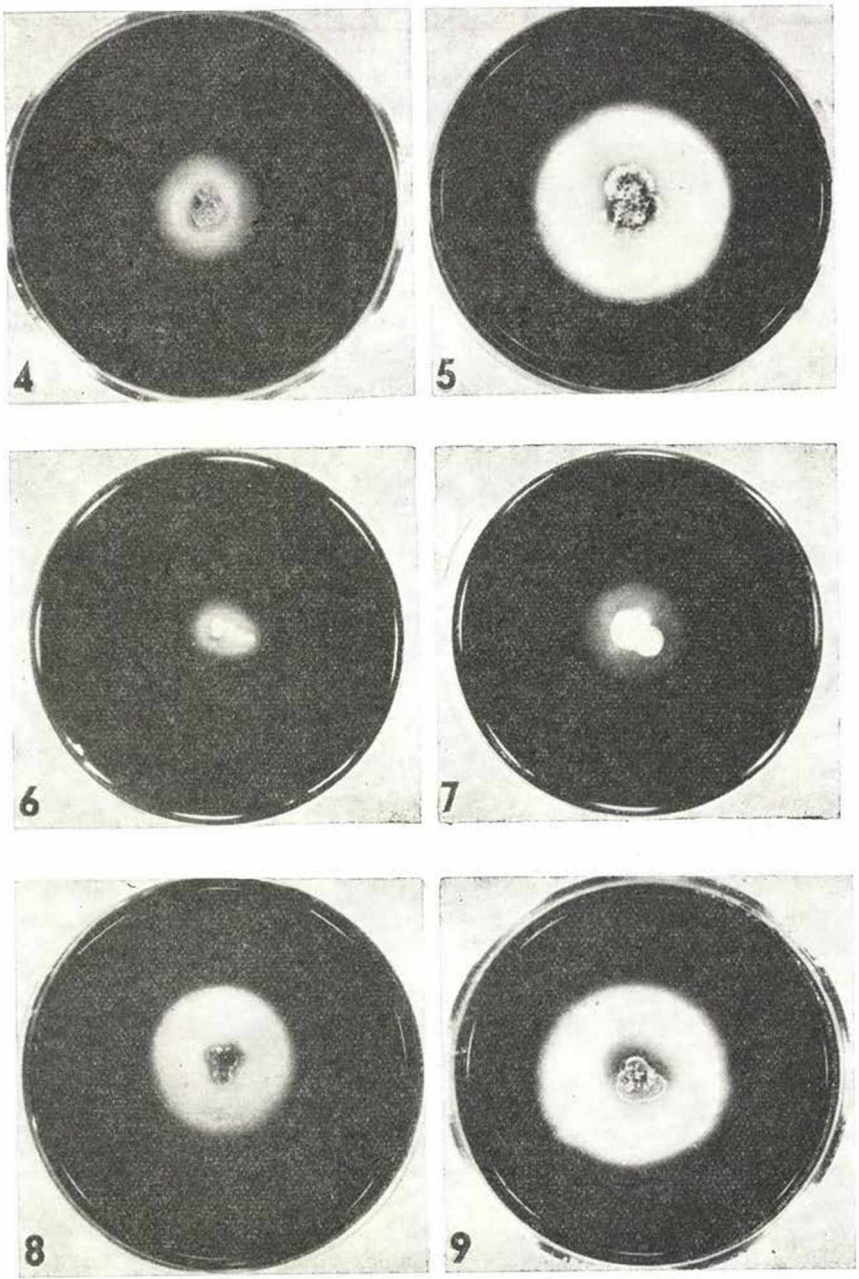

Fig. 4 – 5. Starch decomposition with strain TA_{29} (at 37° and 50°C, respectively)
Fig. 6 – 7. Starch decomposition with strain TA_{60} (at 37° and 50°C, respectively)
Fig. 8 – 9. Starch decomposition with strain TA_{6} (at 37° and 50°C, respectively)

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